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Ghosts from the past: even comprehensive sampling of the native range may not be enough to unravel the introduction history of invasive species—the case of *Acacia dealbata* invasions in South Africa

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PREMISE OF THE STUDY: Knowledge about the introduction history (source(s), number and size of introduction events) of an invasive species is a crucial prerequisite to understand invasion success and to facilitate effective and sustainable management approaches, especially for effective biological control. We investigated the introduction history of the Australian legume tree *Acacia dealbata* in South Africa. Results of this study will not only provide critical information for the management of this species in South Africa, but will also broaden our overall knowledge on the invasion ecology of this globally important invasive tree.

METHODS: We used nuclear microsatellite markers to compare the genetic diversity and structure between 42 native Australian and 18 invasive South African populations and to test different and competing introduction scenarios using Approximate Bayesian Computation analyses.

KEY RESULTS: Australian populations were characterized by two distinct genetic clusters, while South African populations lacked any clear genetic structure and showed significantly lower levels of genetic diversity compared to native range populations. South African populations were also genetically divergent from native populations and the most likely introduction scenario indicated an unknown source population.

CONCLUSIONS: Although we cannot definitely prove the cause of the observed genetic novelty/diversification in South African *Acacia dealbata* populations, it cannot be attributable to insufficient sampling of native populations. Our study highlights the complexity of unravelling the introduction histories of commercially important alien species.

KEY WORDS Fabaceae; genetic diversity; genetic structure; invasion history; microsatellites; tree invasions.

Invasive species have severe impacts on national economies, human livelihoods, as well as biodiversity, and their management is expensive (Pimentel, 2002; Richardson et al., 2015; Early et al., 2016). Despite recent advances in invasion science there are still fundamental knowledge gaps which hamper the complete understanding of invasion processes and the more efficient management of invasive species (Packer et al., 2017). For example, South Africa is currently experiencing one of the worst droughts in centuries while invasive alien trees and shrubs are significantly reducing streamflow from catchments in semi-arid areas (Le Maitre et al., 2016; van Wilgen, 2016). Invasive Australian acacias (genus *Acacia* s.s. formerly *Acacia* subgenus Phyllodineae, family Fabaceae) are major contributors to the reduction of this critical ecosystem service (Le Maitre et al., 2016). While many types of control measures are being implemented to reduce the extent and impacts of invasive plants in South Africa, including biological control (Zachariades et al., 2017), substantial challenges remain (Richardson and Kluge, 2008; van Wilgen and Wannenburgh, 2016).

Knowing your enemy is a first and crucial step in the effective management of invasive species (Pyšek et al., 2013). Accurate species identity and knowledge of its introduction history (source(s), number and size of introductions, residence time, etc.) are important considerations for the implementation of management strategies (Le Roux and Wieczorek, 2009). For example, the effectiveness of biological control will benefit from precise information on the taxonomic identity and native range provenance of the target species (Moran et al., 2013; Pyšek et al., 2013). Molecular tools are especially helpful for resolving some of these issues, e.g., reconstructing introduction histories (see e.g., Cristescu, 2015) and determining source regions (see e.g., Gaskin et al., 2013; Moody et al., 2016). These approaches can also provide information on evolutionary mechanisms underlying invasiveness (e.g., rapid evolution; Zenni et al., 2014), the role of spatial sorting during range expansions (Colautti and Lau, 2015), and the occurrence of admixture or hybridization (Tiébré et al., 2007; Hirsch et al., 2017a). However, using genetic information as proxies for sampling effects (e.g., founder events and genetic bottlenecks), demographic dynamics (e.g., rapid range expansions and spatial sorting), or local adaptation, has drawbacks. A major obstacle in these studies is whether sampling intensity accurately reflects actual ranges of study species and captures most genetic variation found within them (Dlugosch and Parker, 2008; Fitzpatrick et al., 2012). This issue is neglected in many studies, with potentially serious consequences for the accuracy of inferences that can be made (Muirhead et al., 2008), especially for taxa with large native distributions.

In this paper we aim to unravel the introduction history of the Australian silver wattle, *Acacia dealbata* Link, in South Africa. In its native range in Australia, the species occurs over a large area that includes tablelands and slopes in the Australian Capital Territory, New South Wales, Victoria, and eastern Tasmania with summer-maximum, uniform, and winter-maximum rainfall regimes (Poynton, 2009; Lorenzo et al., 2010; CABI, 2018). Silver wattle is a fast-growing tree and reaches reproductive maturity after four to five years (Stelling, 1998). The species has been widely introduced around the world for multiple purposes (e.g., forestry, horticulture, perfume production), and is now a widespread and globally important invader (notably in Chile, Portugal, South Africa, and Spain) (Poynton, 2009; Lorenzo et al., 2010; Richardson et al., 2011). In its invasive ranges *A. dealbata* displaces and changes native vegetation by forming dense mono-specific thickets and altering soil properties due to the release of allelopathic compounds and the species' ability to fix atmospheric nitrogen (Poynton, 2009; Lorenzo et al., 2013). In South Africa, *A. dealbata* invades predominantly riparian habitats in the north-eastern summer rainfall regions of the country (Rouget et al., 2004; Holmes et al., 2005). *Acacia dealbata* was introduced in South Africa in the mid-19th century and by 1886 it was cultivated over large areas in the KwaZulu-Natal province (Poynton, 2009). A secondary introduction from Italy of 4.5 kg of seeds (ca. >350,000 seeds) in 1909 has also been documented (Poynton, 2009). Tracing the exact origins of *A. dealbata* introductions in South Africa is complicated by the fact that seeds were commercially distributed for both profit and non-profit plantations, often without exact information on their provenances (Poynton, 2009). Consequently, to our knowledge, no exact information is available on the direct native source(s) of South African germplasm, other than that it may have included introductions from both mainland Australia and Tasmania (Wattle Research Institute, 1975; Poynton, 2009). A recent study found that *A. dealbata* populations from mainland Australia and Tasmania represent two distinct genetic clades (Hirsch et al., 2018), implying that admixture may be occurring in South Africa.

We used microsatellite data from 18 South African and a previously generated dataset for 42 native *A. dealbata* populations which were sampled across the species' entire native range (Hirsch et al., 2018) with the aim of: (1) gaining information on the genetic diversity and structure present in *A. dealbata*'s invasive range in South Africa; and (2) shedding light on the introduction/invasion history

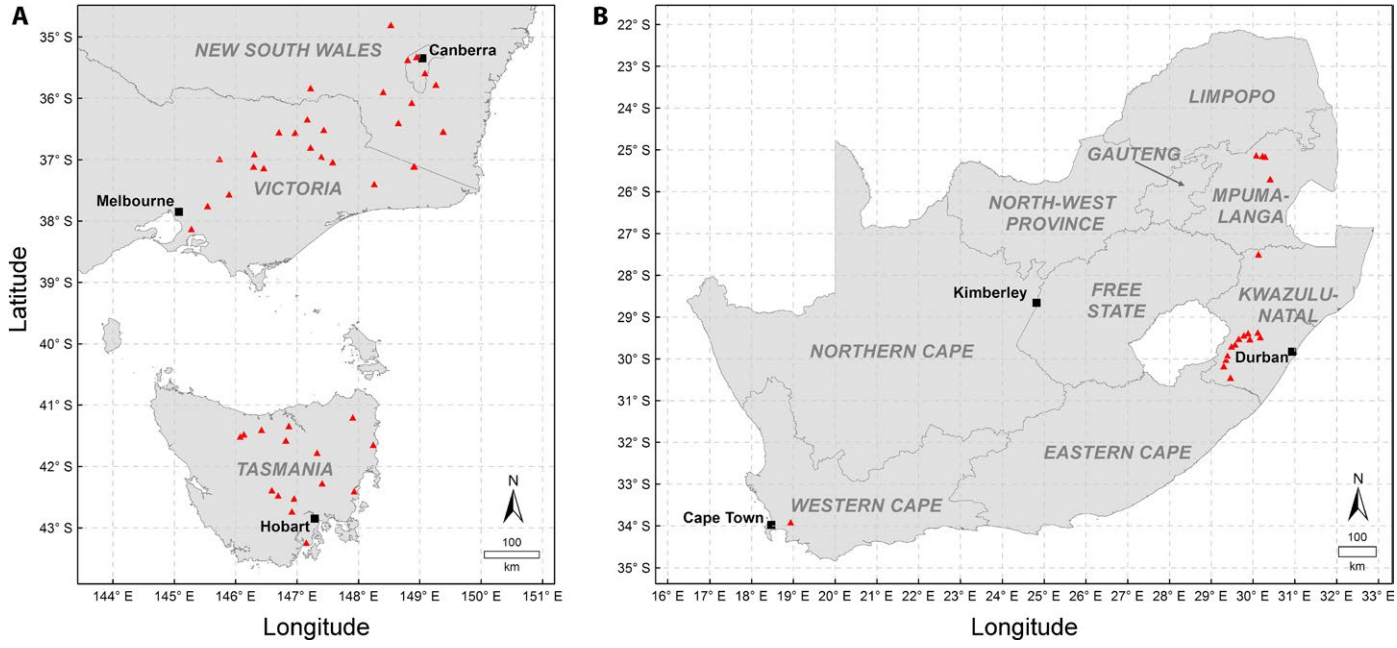

FIGURE 1. Locations of the native Australian (A) and invasive South African (B) populations of *Acacia dealbata* (shown as red triangles) included in this study.

of *A. dealbata* in South Africa by testing our data against different introduction scenarios using Approximate Bayesian Computation.

MATERIALS AND METHODS

Sampling, DNA extraction and genotyping

In addition to the genotype data of native *A. dealbata* populations in Australia from Hirsch et al. (2018) (Fig. 1A), we included data from 18 populations in the species' invasive range in South Africa (Fig. 1B). For these, fresh and healthy leaves were sampled from 20 randomly chosen individuals per population (total n = 360). Leaf material was dried and stored on silica gel until DNA extraction. The sampling area in the native range was based on distribution records (worldwidewattle.com; florabank.org.au; environment.gov.au/science/abrs/online-resources/flora-of-australia-online). While planted stands of native flora are potentially difficult to distinguish from naturally occurring populations, obvious re-vegetation projects (along main roadsides and freeways) were avoided, and sampling focused on what appeared to be natural populations which were frequently off the beaten track, thus providing a representative sample across the species' native range.

DNA was extracted using the cetyltrimethylammonium bromide (CTAB) method (Doyle and Doyle, 1990) with some modifications. We added 0.2 M sodium sulphite to the extraction buffer to minimize the degradation of DNA (Byrne et al., 2001). All DNA extractions were diluted to a concentration of 100 ng/µl.

We amplified ten nuclear microsatellites in two multiplex PCR reactions (for further details see Hirsch et al., 2018 and Appendix S1). All PCR reactions were carried out in a volume of 10 µl containing 2 µl template DNA (100ng/µl), 5 µl KAPA2G Fast Multiplex Mix (Kapa Biosystems, Cape Town, South Africa), 1 µl primer mix of the corresponding multiplex set (Thermo Scientific, Waltham, Massachusetts, USA), 2 µl purified H_2O. PCR cycling was performed in a MultiGene OptiMax thermal cycler (Labnet International, Edison, New Jersey, USA) with an initial denaturation of 95°C for 3 min, followed by 30 cycles of denaturation at 95°C for 15 sec, annealing at 60°C for 30 sec, elongation at 72°C for 30 sec, and a final elongation at 72°C for 10 min. Each 96-well PCR plate contained 92 samples plus three randomly selected replicate samples and one negative control (H_2O). Gel capillary electrophoretic separation of the amplified fragments was carried out at the Central Analytical Facility, Stellenbosch University, Stellenbosch, South Africa. Semi-automatic genotype scoring of all samples (i.e., samples from native and invasive populations) was performed using the GeneMarker software (version 2.6.4; SoftGenetics LLC, State College, Pennsylvania, USA) by applying marker panels to call alleles. This was followed by manual checking of all scored alleles. Genotypes were obtained for a total of 292 South African individuals. These were combined with the native range genotypes from Hirsch et al. (2018), resulting in a final dataset of 1057 samples, representing 42 native Australian and 18 South African populations (Table 1).

Dataset characteristics and genetic diversity

Micro-Checker version 2.2 (Van Oosterhout et al., 2004) was used to check for the presence of null alleles and scoring errors in the dataset. The frequency of null alleles was estimated at each locus and population following the expected maximization method as implemented in the software FreeNA (Chapuis and Estoup, 2007). FreeNA was further employed for the calculation of uncorrected and corrected (i.e., excluding null alleles; so-called ENA method as described in Chapuis and Estoup, 2007) estimates of pairwise F_{ST} values (Weir, 1996). All loci were tested for allele frequency departures from Hardy-Weinberg equilibrium (HWE) expectations using the packages adegenet version 2.0.1 (Jombart, 2008) and pegas version 0.9 (Paradis, 2010) in R (R Core Team, 2016).

To evaluate the genetic diversity of each population, the package diveRsity (Keenan et al., 2013) was used to calculate allelic richness (A_R), observed and expected heterozygosity (H_O and H_E). For the A_R calculations, a rarefaction correction was applied to account for different sample sizes among populations. The package diveRsity was also used for the calculation of inbreeding coefficients (F_{IS}) for each population.

To assess whether genetic diversity indices (i.e., A_R, H_O, and H_E) differed between the native and invasive populations of *A. dealbata*, we carried out analyses of variance (ANOVA) in R (R Core Team, 2016). For these analyses, we considered the previously described population genetic structure among the native populations (Hirsch et al., 2018). That is, native populations were assigned to two groups: (1) 'NAT1' (corresponding to Cluster 1 in Hirsch et al. (2018), containing the majority of Australian mainland populations); and (2) 'NAT2' (i.e., Cluster 2 in Hirsch et al. (2018), containing all Tasmanian and some populations from the southernmost Australian mainland) (Table 1). Another group (RSA) contained all South African populations. All three genetic diversity indices fulfilled the assumptions of ANOVA (normality and homoscedasticity). In the cases of significant results, Tukey's HSD tests were used to determine which genetic groups (NAT1, NAT2, and RSA) differed significantly from each other.

Genetic structure and variation

We applied Bayesian assignment tests to investigate the genetic structure among populations within the native and invasive range of *A. dealbata* as implemented in STRUCTURE version 2.3.4 (Pritchard et al., 2000). A range of possible genetic cluster values (*K* values), from 1 to 30, was tested by using an admixture model with correlated allele frequencies, 100,000 burn-in iterations, 500,000 Markov Chain Monte Carlo repetitions, and 20 iterations for each value of *K*. The delta *K* method of Evanno et al. (2005) was applied with the online software STRUCTURE HARVESTER (version 0.6.94; Earl and vonHoldt, 2012) to obtain the optimum number of clusters. CLUMPP (version 1.1.2; Jakobsson and Rosenberg, 2007) and DISTRUCT (version 1.1; Rosenberg, 2004) was used for a graphical display of STRUCTURE results. A second STRUCTURE analysis was done using the same protocol by considering only populations from the invasive range in South Africa and by testing for *K* ranging from 1 to 18. We also tested whether the extent of genetic differentiation between pairs of populations (i.e., pairwise F_{ST} values) differs between the native range and the invasive range of *A. dealbata* using a Kruskal-Wallis rank sum test. As an additional approach to explore and visualize population genetic structure, we performed a principal coordinate analysis (PCoA) using the vegan R package (version 2.4-2; Oksanen et al., 2017) based on the uncorrected genetic distances (Cavalli-Sforza and Edwards, 1967) calculated with FreeNA.

We tested for isolation-by-distance (IBD) among *A. dealbata* populations in South Africa using a Mantel test (Mantel, 1967). This has been previously done for native populations (Hirsch et al., 2018). For IBD, genetic distances between populations were

TABLE 1. Populations of *Acacia dealbata* investigated in this study. Native populations from Tasmania (TAS) and mainland Australia (AUS) were previously described and analysed by Hirsch et al. (2018). For each population, the location (in decimal degrees; Lat = latitude; Long = longitude), number of samples (N), allelic richness (A_R), observed (H_O) and expected (H_E) heterozygosity, and the inbreeding coefficient (F_{IS}) are provided. STRUCTURE cluster indicates the clusters to which populations were allocated for the analyses of variance (ANOVA) and the Approximate Bayesian Computation (ABC) analyses. Please note that A_R values reported here for native populations differ slightly from those reported by Hirsch et al. (2018) due to rarefaction.

Population ID	Region	Lat	Long	N	A_R	H_O	H_E	F_{IS}	STRUCTURE cluster
NATIVE RANGE									
TAS_1	TAS	−41.51	146.08	19	3.353	0.458	0.463	−0.010	NAT2
TAS_2	TAS	−41.47	146.13	18	3.541	0.441	0.451	0.052	NAT2
TAS_3	TAS	−41.40	146.42	18	3.324	0.488	0.463	−0.074	NAT2
TAS_4	TAS	−41.57	146.82	18	3.251	0.402	0.467	0.137	NAT2
TAS_5	TAS	−41.78	147.33	14	3.192	0.473	0.489	0.056	NAT2
TAS_6	TAS	−42.27	147.41	20	2.757	0.596	0.507	−0.211	NAT2
TAS_7	TAS	−42.52	146.95	19	1.641	0.474	0.265	−0.556	NAT2
TAS_8	TAS	−42.47	146.70	17	2.817	0.492	0.423	−0.115	NAT2
TAS_9	TAS	−42.39	146.59	17	3.761	0.511	0.530	0.031	NAT2
TAS_10	TAS	−42.73	146.92	19	2.992	0.510	0.415	−0.156	NAT2
TAS_11	TAS	−43.24	147.15	19	3.490	0.491	0.534	0.054	NAT2
TAS_12	TAS	−42.40	147.93	20	2.403	0.417	0.339	−0.181	NAT2
TAS_13	TAS	−41.65	148.24	17	3.543	0.529	0.522	−0.002	NAT2
TAS_14	TAS	−41.20	147.91	17	3.724	0.524	0.557	0.079	NAT2
TAS_15	TAS	−41.34	146.87	20	3.292	0.472	0.493	0.037	NAT2
AUS_1	AUS	−38.13	145.28	18	3.294	0.497	0.513	0.039	NAT2
AUS_2	AUS	−37.75	145.55	20	3.387	0.530	0.510	−0.051	NAT2
AUS_3	AUS	−37.56	145.89	19	4.009	0.558	0.566	0.015	NAT2
AUS_4	AUS	−36.99	145.74	19	2.224	0.485	0.335	−0.362	NAT1
AUS_5	AUS	−37.14	146.46	18	4.016	0.529	0.562	0.095	NAT1
AUS_6	AUS	−37.11	146.30	19	2.499	0.495	0.435	−0.130	NAT1
AUS_7	AUS	−36.91	146.30	19	3.040	0.533	0.466	−0.124	NAT1
AUS_8	AUS	−36.55	146.71	18	3.825	0.549	0.593	0.055	NAT1
AUS_9	AUS	−36.34	147.17	18	2.897	0.415	0.425	0.008	NAT1
AUS_10	AUS	−36.51	147.44	19	3.011	0.489	0.450	−0.014	NAT1
AUS_11	AUS	−36.56	146.97	18	3.541	0.528	0.517	−0.012	NAT1
AUS_12	AUS	−36.80	147.22	17	2.711	0.518	0.408	−0.194	NAT1
AUS_13	AUS	−36.95	147.40	18	2.951	0.526	0.432	−0.185	NAT1
AUS_14	AUS	−37.04	147.58	19	3.048	0.489	0.466	0.001	NAT1
AUS_15	AUS	−37.39	148.26	17	3.620	0.499	0.506	0.012	NAT1
AUS_16	AUS	−35.89	148.41	19	1.977	0.603	0.371	−0.581	NAT1
AUS_17	AUS	−36.07	148.87	17	1.920	0.400	0.279	−0.214	NAT1
AUS_18	AUS	−36.40	148.65	18	3.217	0.454	0.464	0.029	NAT1
AUS_19	AUS	−37.11	148.90	20	2.096	0.481	0.403	−0.202	NAT1
AUS_20	AUS	−37.11	148.91	17	2.466	0.290	0.320	0.128	NAT1
AUS_21	AUS	−36.54	149.38	19	3.269	0.484	0.509	0.000	NAT1
AUS_22	AUS	−35.78	149.26	18	3.193	0.585	0.518	−0.109	NAT1
AUS_23	AUS	−35.59	149.09	19	2.809	0.558	0.469	−0.098	NAT1
AUS_24	AUS	−35.32	148.95	18	2.656	0.556	0.460	−0.224	NAT1
AUS_25	AUS	−35.37	148.80	18	3.274	0.443	0.478	0.067	NAT1
AUS_26	AUS	−34.80	148.53	17	3.398	0.473	0.524	0.095	NAT1
AUS_27	AUS	−35.83	147.22	17	2.696	0.486	0.442	−0.111	NAT1
				Mean	**3.051**	**0.494**	**0.460**	**−0.070**	
				Standard deviation	**0.565**	**0.058**	**0.075**	**0.160**	
INVASIVE RANGE									
RSA_1	RSA	−33.91	18.95	19	2.308	0.368	0.353	−0.030	RSA
RSA_2	RSA	−27.50	30.13	18	2.585	0.418	0.435	0.021	RSA
RSA_3	RSA	−25.70	30.42	9	3.000	0.488	0.474	−0.014	RSA
RSA_4	RSA	−30.45	29.46	10	2.317	0.410	0.341	−0.159	RSA
RSA_5	RSA	−30.17	29.31	11	2.351	0.705	0.458	−0.484	RSA
RSA_6	RSA	−30.02	29.36	16	2.339	0.493	0.446	−0.098	RSA
RSA_7	RSA	−29.92	29.39	11	1.987	0.627	0.363	−0.658	RSA
RSA_8	RSA	−29.70	29.49	20	2.581	0.405	0.415	0.085	RSA
RSA_9	RSA	−29.65	29.58	17	2.075	0.457	0.362	−0.230	RSA
RSA_10	RSA	−29.52	29.66	20	2.653	0.479	0.451	−0.039	RSA

(Continued)

TABLE 1. (Continued)

Population ID	Region	Lat	Long	N	A_R	H_O	H_E	F_{IS}	STRUCTURE cluster
RSA_11	RSA	−29.43	29.79	19	2.395	0.326	0.354	0.065	RSA
RSA_12	RSA	−29.38	29.89	20	2.831	0.418	0.418	0.021	RSA
RSA_13	RSA	−29.48	30.18	18	2.729	0.407	0.462	0.179	RSA
RSA_14	RSA	−29.37	30.12	12	2.415	0.485	0.416	−0.145	RSA
RSA_15	RSA	−29.53	29.93	18	2.277	0.447	0.429	−0.025	RSA
RSA_16	RSA	−25.13	30.08	20	2.315	0.389	0.390	0.000	RSA
RSA_17	RSA	−25.14	30.23	18	1.758	0.506	0.277	−0.521	RSA
RSA_18	RSA	−25.16	30.30	16	2.517	0.441	0.404	−0.022	RSA
				Mean	*2.413*	0.459	0.403	−0.114	
				Standard deviation	*0.300*	0.090	0.052	0.225	

represented as linearized pairwise F_{ST} values (i.e., F_{ST} / 1-F_{ST}) and geographic distances were calculated from the GPS coordinates for each population with the software Geographic Distance Matrix Generator (version 1.2.3; Erst, 2017). The IBD test was carried out in the vegan R package with 9999 permutations.

To access the genetic variation between and among populations from the native Australian and invasive South African range, a hierarchical analysis of molecular variance (AMOVA; Excoffier et al., 1992) was performed with populations nested within ranges. The AMOVA was conducted with 9999 permutations in the poppr R package (version 2.3.0; Kamvar et al., 2014, 2015).

Inferring the introduction history of *Acacia dealbata* in South Africa

To better understand the introduction history of *A. dealbata* in South Africa, Approximate Bayesian Computation (ABC) analyses (Beaumont et al., 2002; Beaumont, 2010) were done using the software DIYABC (version 2.1.0; Cornuet et al., 2015). This approach allows the simulation of a large number of genetic datasets for different potential introduction scenarios that are compared with the observed dataset to identify the most likely scenario (Beaumont, 2010; Chau et al., 2015; Barker et al., 2017). To define potential native source regions in Australia, native populations were pooled into population genetic clusters identified by Hirsch et al. (2018). Because of the lack of a clear genetic structure in South Africa (see Results), all non-native populations were pooled within a single genetic cluster (i.e., 'RSA'). Despite comprehensive sampling in the native range, we cannot, with 100% certainty, exclude the possibility that we did not sample Australian genetic groups from which germplasm were introduced to South Africa. We therefore included an overarching native population 'NAT0' which links the two native range clusters into one, but also allowed us to include a potentially unsampled source as the provenance of South African populations. We also considered the possibility that South African populations might represent admixed populations of both native genetic clusters, and the possibility of multiple introductions. The following competing introduction scenarios were tested: (1) RSA has direct ancestral origin from NAT1; (2) RSA has direct ancestral origin from NAT2; (3) RSA has direct ancestral origin from an unknown source (NAT0) which is related to NAT1 and NAT2; (4) RSA originated from an unsampled non-native population (i.e., ghost population) with admixed ancestry of both NAT1 and NAT2; (5) RSA has ancestral origin from NAT1 after multiple introductions; and (6) RSA has ancestral origin from NAT2 after multiple introductions (Appendix S2).

As an initial step, following the recommendations by Bertorelle et al. (2010), we conducted preliminary DIYABC runs to optimize prior estimates. For the final analysis, 1×10^6 datasets were simulated for each scenario using the high-performance computation cluster at Stellenbosch University's Central Analytical Facilities' (HPC2; http://www.sun.ac.za/hpc) and by applying the prior distributions of parameters and parameter rules reported in Appendix S3. Based on the historical information that *A. dealbata* was introduced to South Africa in the middle of the 19th century (Poynton, 2009) and given its minimum generation time of four to five years (Stelling, 1998), we assumed that the invasion in South Africa is not older than 45 generations (Appendix S3). We applied a generalized stepwise mutation model for the ABC approach for which mean number of alleles, mean genetic diversity (Nei, 1987), mean allele size variance, mean Garza-Williamson's M (Garza and Williamson, 2001; Excoffier et al., 2005), F_{ST} (Weir and Cockerham, 1984), shared allele distance (Chakraborty and Jin, 1993) and $(\delta\mu)^2$ genetic distance (Goldstein et al., 1995) were considered for the summary statistics. The posterior probabilities of the competing scenarios were compared using logistic regression on the 1% of simulated data sets closest to the observed data set (Cornuet et al., 2010). For each parameter, posterior distributions were estimated under the best scenario (see Results) by applying a local linear regression on the 1% closest simulated data sets (i.e., 10,000 data sets per scenario) with logit transformation. The precision of parameter estimations was assessed by calculating the median of the absolute deviation (RMedAD) and the median relative bias (MedRB) on 500 test data sets for the most likely scenario (Cornuet et al., 2010).

Additional validation of the ABC results was done by using the 'confidence in scenario choice' function implemented in the DIYABC software and following the approach described by Cornuet et al. (2010). This allowed us to estimate the type I error (i.e., the probability that a scenario is rejected when it is true) and type II error (i.e., the mean proportion of instances in which the scenario with the highest posterior probability was incorrectly identified as the most likely scenario, calculated across the other competing scenarios) for the scenario which revealed the highest posterior probability (see Results). A set of 100 independent data sets and the logistic regression approach were used for these error estimations. We also used the 'model checking' option of DIYABC to test the ability (i.e., adequacy) of the most likely scenario to simulate data sets similar to the observed data set (Cornuet et al., 2010). For this, 1000 data sets were simulated from the posterior distribution of the parameters of the corresponding scenario; a set of summary

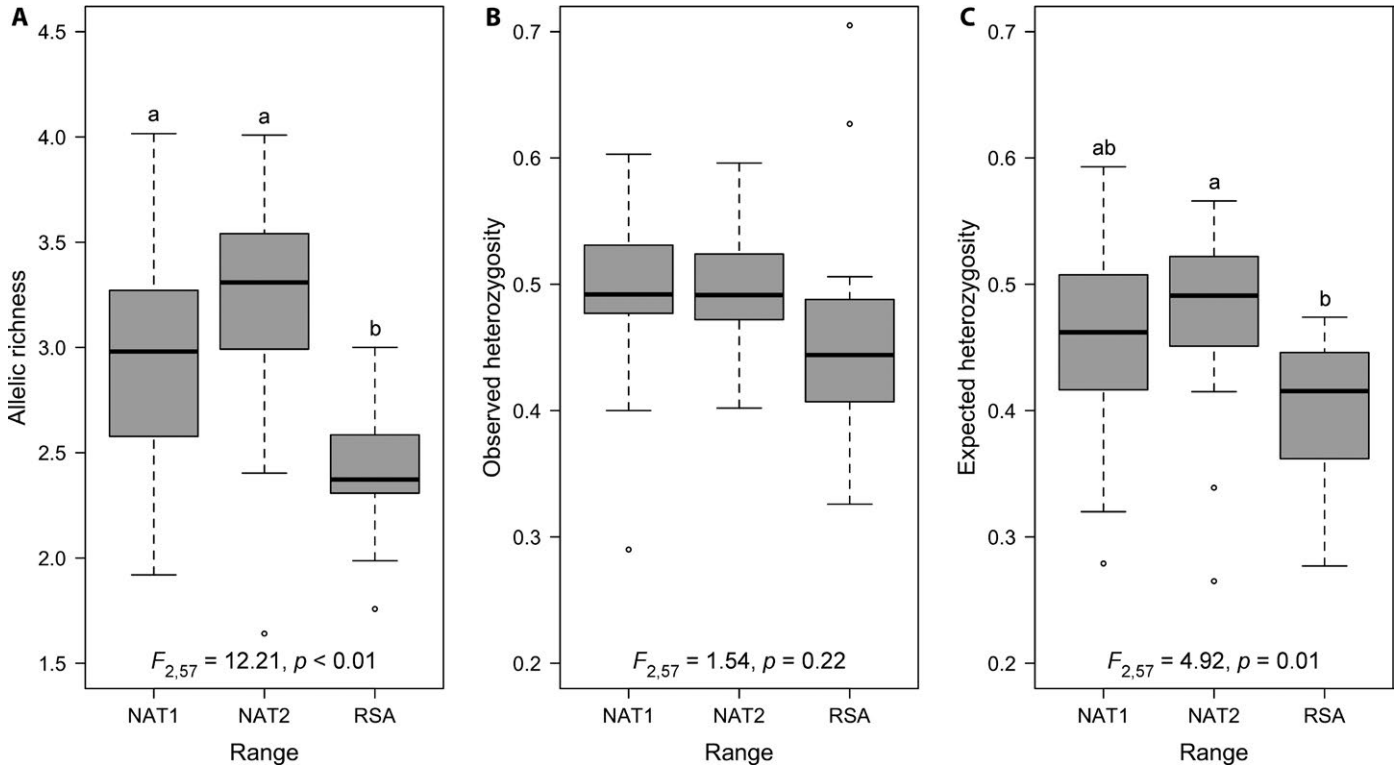

FIGURE 2. Comparisons of genetic diversity measures between the native Australian and invasive South African populations of *Acacia dealbata* for: (A) allelic richness, (B) observed heterozygosity, and (C) expected heterozygosity. Native populations are represented by two genetic clusters according to Hirsch et al. (2018) (NAT1 = mainly Australian mainland populations; NAT2 = Tasmanian populations and southern most mainland populations). Invasive populations are represented by one genetic cluster (RSA). Different letters above boxplots indicate significant differences (ANOVA, $P < 0.05$; Tukey's post hoc test) between the corresponding groups.

statistics different to the one for previous inferential steps were used to avoid overestimating the fit of a scenario (Cornuet et al., 2010).

RESULTS

Dataset characteristics and genetic diversity

No scoring errors caused by stuttering were identified for the final dataset and all loci were polymorphic with between 5 and 17 alleles per locus (Appendix S1). All loci showed significant departures from the HWE expectations for at least four populations, and 166 out of 600 locus-by-population comparisons showed significant departures (Appendices S1 and S4). A low mean null allele frequency of 0.025 was identified but no significant difference could be detected between uncorrected and ENA-corrected pairwise F_{ST} values (Kruskal-Wallis chi-square = 4, $P = 0.41$). All further analyses were therefore performed without correcting for null alleles.

The ANOVA approach revealed significant differences in A_R ($F_{2,57} = 12.21$, $P < 0.01$) and H_E ($F_{2,57} = 4.92$, $P = 0.01$), but not in H_O ($F_{2,57} = 1.54$, $P = 0.22$) (Fig. 2). Significantly lower A_R was found in the invasive range than in in both native genetic clusters (Fig. 2). For H_E, only populations from the NAT2 cluster showed significantly higher values than the invasive populations (Fig. 2). No differences between the two native genetic clusters were identified for both A_R and H_E (Fig. 2). F_{IS} values showed that all populations are characterized by very low or no inbreeding (Table 1).

Genetic structure and variation

Two distinct genetic clusters were identified by Bayesian assignment tests for the full dataset, roughly corresponding to one native and one invasive cluster (Fig. 3, Appendix S5). Three invasive populations (i.e., RSA_13; RSA_17; and RSA_18), however, showed levels of admixture between these two clusters (Fig. 3). A strong signal was also detected for $K = 3$ (Appendix S5) which confirmed a further division of native population into the two clusters previous identified by Hirsch et al. (2018). When considering only invasive populations from South Africa, the STRUCTURE approach revealed only very weak (i.e., approximately 10 times lower than for the overall dataset) and unclear patterns for delta K (Appendix S5). No clear genetic structure could thus be detected among the invasive populations of *A. dealbata* in South Africa. This lack of genetic structure was reflected by significantly lower pairwise F_{ST} values between pairs of invasive populations compared to native populations (Kruskal-Wallis chi-square = 41.16, $P < 0.01$; Appendix S6).

The PCoA approach reflected the substantial genetic differentiation between invasive and native range populations and the gradual differentiation between native populations from the Australian mainland and Tasmania (Appendix S7). No genetic IBD was found among South African populations of *A. dealbata* (Mantel $r = 0.24$, $P = 0.10$). According to the AMOVA results, the highest genetic variation was detected within populations (66.6%), followed by 23.1% between populations and 10.3% between the two ranges (Appendix S8).

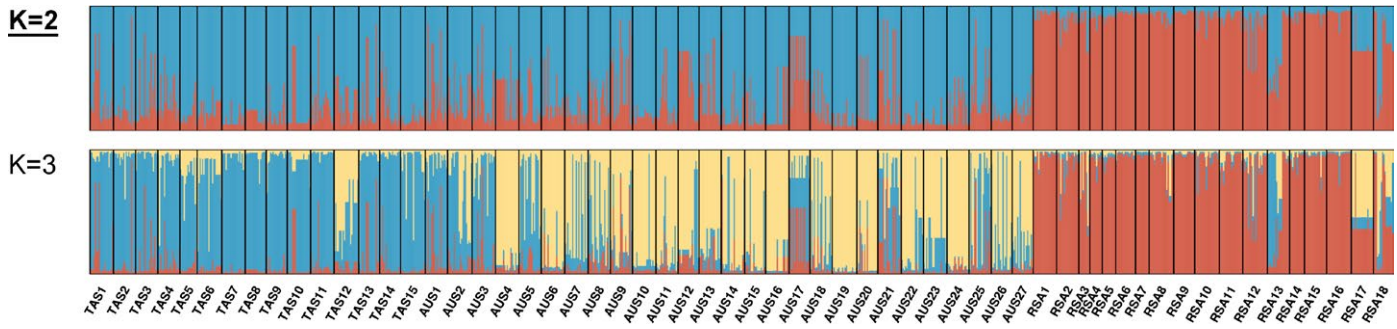

FIGURE 3. STRUCTURE bar plots for two and three genetic clusters (i.e., $K = 2, 3$) among the investigated native Australian and invasive South African populations of *Acacia dealbata*. The delta *K* method following Evanno et al. (2005) revealed K=2 as optimal genetic structure but also showed a strong signal for *K*=3 (Appendix S5). The population ID's beneath the bar plots refer to the ID's listed in Table 1.

Introduction history of invasive populations in South Africa

The ABC analysis supported the scenario that South African *A. dealbata* populations originated from an unknown ('ghost') source, rather than directly from the two included native range genetic clusters (NAT1 and NAT2) with the highest posterior probability (scenario 3: $p = 0.418$; 95% CI = 0.381–0.455; Fig. 4). The posterior distributions of parameters estimated under the three most-likely scenarios indicated a mean time of introduction 26.3 generations (i.e., approximately 105–132 years) ago, associated with a short (mean: 2.7 generations) and a relatively strong (i.e., mean effective size of founder population = 468 individuals) bottleneck event (Appendix S3). The majority of RMedAD values for the scenario 3 parameter were estimated with high confidence. Some of the lower MedRB values were revealed for RSA, RSAb and t_{inv}, which were therefore plausibly estimated (Appendix S3). Scenario 3 was characterized by very low Type I (0.01) and Type II (0.006) errors. Results of the adequacy test via the model checking function in DIYABC showed no significant deviation between observed and simulated summary statistics for scenario 3; indicating that the posterior distributions of this scenario are well corroborated by the observed data and that this scenario correctly explains the 'real' observed data (Appendix S9).

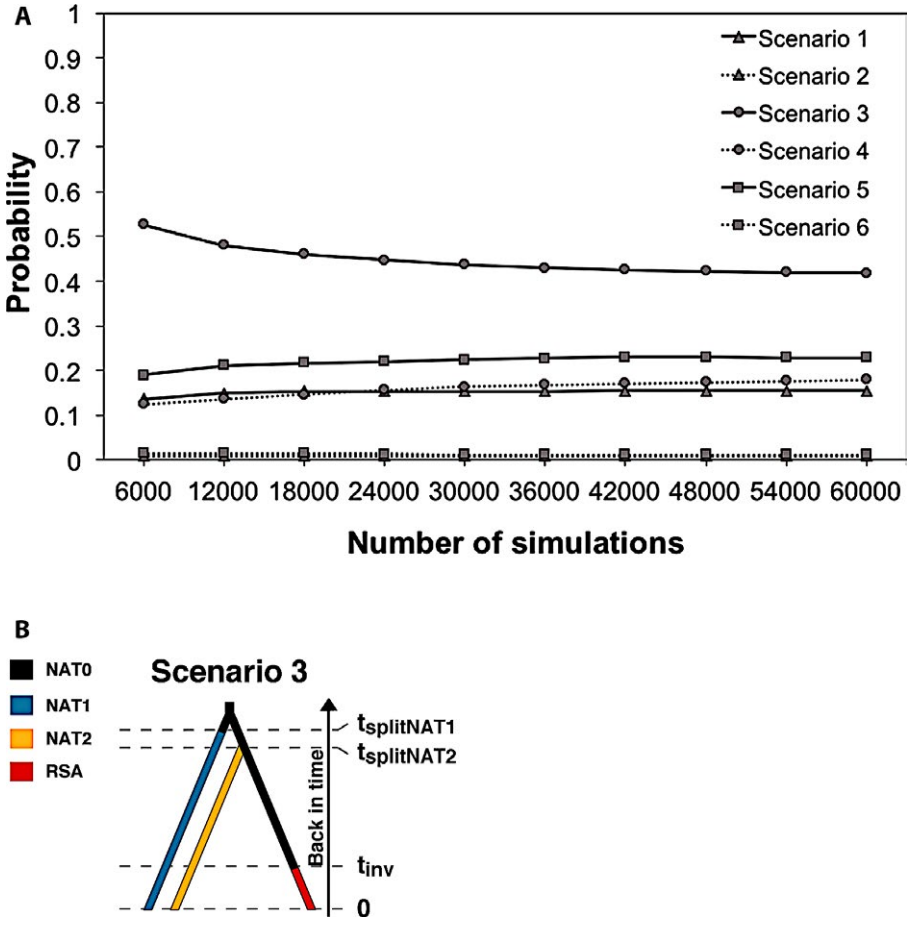

FIGURE 4. Results of the Approximate Bayesian Computation analyses (ABC) to test different introduction scenarios for *Acacia dealbata* populations in South Africa: (A) Posterior probabilities for each of the six tested scenarios estimated via the logistic regression approach in the software DIYABC (Cornuet et al., 2015); and (B) Illustration of Scenario 3 which showed the highest posterior probability. Each founding event of a new population, indicated by a change of color in the scenario pathway (see details in Appendix S3). Illustrations of all six competing scenarios are shown in Appendix S2.

DISCUSSION

We found that invasive *A. dealbata* populations in South Africa differ substantially in their genetic composition from a wide and representative geographic sample of native Australian populations, with the latter displaying significantly higher genetic diversity and structure. This conforms to the common assumption that invasive populations often have reduced genetic diversity as a result of founding events (Dlugosch and Parker, 2008), as is often evident for invasive

trees (e.g., *Olea europaea* L. subsp. *cuspidata* (Wall. & G.Donn) Cif. [Oleaceae]; Besnard et al., 2007). The most noticeable change in genetic diversity in the South African populations was a sharp reduction in allelic richness, a diversity metric more sensitive to bottleneck events of short duration than other metrics such as heterozygosity (Allendorf, 1986), an inference supported by our ABC results. Theory predicts that a reduction of allelic richness following bottleneck events can hamper the ability of a population to adapt and survive over the long term (Allendorf, 1986; Greenbaum et al., 2014). It remains to be determined whether adaptive genetic diversity of South African *A. dealbata* populations shows a similar reduction as was observed for neutral genetic diversity here. Despite reduced genetic diversity, South African populations showed low levels of inbreeding, suggesting either that these populations did not experience a genetic bottleneck or, more likely, may have recovered from such an event following introduction.

The most likely introduction history scenario supported by our ABC analyses was that invasive populations in South Africa originated from an unknown source, which links the ancestry between invasive and native range populations of *A. dealbata*. This is also in line with our previous phylogeographic research that identified unique haplotypes in South Africa that were not present in Australian populations (Hirsch et al., 2017b). Together with the population genetic clustering retrieved here (Fig. 3), these findings indicate that the un-sampled population that gave rise to South African *A. dealbata* populations is genetically distinct from the two native range genetic clusters sampled here. This is surprising, since our sampling of the native range was comprehensive; it is unlikely that including additional native range samples would have identified additional genetic clusters to the two identified here (i.e., clusters NAT1 and NAT2). Consequently, even if the particular native source population of invasive *A. dealbata* populations was not sampled (e.g., because it is now extinct), we would have expected to identify at least some degree of relatedness between invasive populations and the native genetic source cluster(s). This assumption is also strengthened by the fact that native populations showed no pronounced genetic sub-structure within the two main clusters (Hirsch et al., 2018). Any further inferences of what may explain the inferred introduction history of *A. dealbata* to South Africa will be speculative. A possible explanation is that the invasive populations in South Africa originated from a cultivated and/or secondary source(s) that may have experienced strong genetic changes associated with genetic bottlenecks, strong drift, and/or selection. Thompson et al. (2012) found invasive populations of another Australian acacia in South Africa (*A. saligna* (Labill.) H.L.Wendl.) to be genetically distinct from all sampled native range populations, but genetically similar to a cultivated population in Australia. They concluded that cultivation was an important contributing factor in shaping the genetic makeup of *A. saligna* populations currently present in South Africa (Thompson et al., 2012). We have documented evidence for at least one secondary introduction of *A. dealbata* from Italy in 1909 (Poynton, 2009). To confirm the occurrence such so-called 'invasive bridgehead scenarios' (Bertelsmeier and Keller, 2018) or secondary introduction events, and their effects on population genetic structure found in South Africa, we recommend including populations from other parts of the global invasive range of *A. dealbata* in future analyses of population genetics of this species. Such a global-scale population genetic analysis of *A. dealbata* would provide invaluable information to gain a more comprehensive picture of the population structure and introduction history of the species (including potential movement between regions outside the native range) in its current adventive range around the globe. It might also be useful to include more South African populations of *A. dealbata* in future genetic investigations to shed more light on the species' invasion history in the country. Although we doubt that such expanded sampling in South Africa would change the overall findings of this study, it could help to gain in-depth knowledge of the post-introduction spread of the species and of gene flow patterns among invasive populations. Future studies may also consider applying maternally inherited chloroplast DNA (cpDNA) markers to further resolve the introduction history of *A. dealbata*. Because of their four-fold smaller effective population size compared to nuclear markers, such cpDNA markers can often detect geographic structure that is not apparent in nuclear DNA (Cavers et al., 2003; Petit et al., 2005). Lastly, the inclusion of genetic material from seed lots distributed for commercial purposes would also help us to better understand the impacts of cultivation on the genetic diversity and structure found in invasive *A. dealbata* populations.

CONCLUSIONS

Our study adds to a growing body of literature that suggests that high genetic diversity is not always a prerequisite for successful invasion of non-native plants, and that invasive populations can differ vastly in their genetic makeup from populations in the native range. Even for well-sampled taxa, resolving introduction histories is challenging, as is the case for *A. dealbata* in South Africa. Such challenges are not unique to the genus *Acacia*; studies on other invasive tree species with complex introduction histories have revealed similar difficulties (e.g., *Ailanthus altissima* (Mill.) Swingle [Simaroubaceae], Kurokochi et al., 2014; *Paraserianthes lophantha* (Willd.) I.C. Nielson [Fabaceae], Le Roux et al., 2011; *Prunus serotina* Ehr. [Rosaceae], Pairon et al., 2010). From a practical point of view, our findings of genetic distinctiveness and unknown origin of invasive *A. dealbata* populations have important consequences for further management efforts. For example, predictions about the species potential range in South Africa might be inaccurate when niche models are based on native occurrences only (Thompson et al., 2012). Further, the response of genetically distinct populations to biological control agents with high host-specificity to native genotypes may be altered and could consequently complicate future biological control attempts (Goolsby et al., 2006; Thompson et al., 2015).

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AUTHOR CONTRIBUTIONS

H.H., J.J.L.R. and D.M.R. designed the study. F.A.C.I., C.K., H.H. and D.M.R. collected samples of *Acacia dealbata*. H.H. did the molecular analysis. H.H. and M.L.C. performed the statistical analyses. H.H. drafted the manuscript with substantial inputs from J.J.L.R. and D.M.R. All authors read and commented on the manuscript.

DATA ACCESSIBILITY

The genotype data generated and analysed in this study is available at FigShare: https://doi.org/10.6084/m9.figshare.7472318.v1.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

APPENDIX S1. Characteristics of the microsatellite loci used in this study.

APPENDIX S2. Schematic representation of all tested ABC scenarios.

APPENDIX S3. Prior and posterior estimates of parameters used for the ABC analysis.

APPENDIX S4. Locus-by-population comparisons for departures from Hardy-Weinberg Equilibrium.

APPENDIX S5. Delta *K* plots.

APPENDIX S6. Comparison of genetic differentiation.

APPENDIX S7. Principal coordinates analysis.

APPENDIX S8. Analysis of molecular variance (AMOVA) results.

APPENDIX S9. Model checking results of the ABC analysis.

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